

PENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

Date of mailing (day/month/year)

28 June 2001 (28.06.01)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

International application No.

PCT/US00/25833

Applicant's or agent's file reference

31978-165733

International filing date (day/month/year)

21 September 2000 (21.09.00)

Priority date (day/month/year)

21 September 1999 (21.09.99)

Applicant

COLLINS, Jerry, M. et al

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

07 March 2001 (07.03.01)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Charlotte ENGER

Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338.83.38

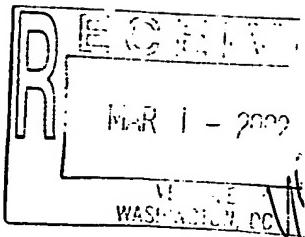
PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

SCHNELLER, M.; HADDAY, K.
Venable
P.O. Box 34385
Washington, DC 20043-9998
ETATS-UNIS D'AMERIQUE

PCT



NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT Rule 71.1)

| | |
|-------------------------------------|------------|
| Date of mailing (day/month/year) | 25.02.2002 |
|-------------------------------------|------------|

| | | |
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| Applicant's or agent's file reference | IMPORTANT NOTIFICATION | |
|---------------------------------------|------------------------|--|

31978-165733

| | | |
|---|--|--|
| International application No. PCT/US00/25833 | International filing date (day/month/year) 21/09/2000 | Priority date (day/month/year) 21/09/1999 |
|---|--|--|

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|--|
| Applicant THE GOVERNMENT OF THE USA, asrepresented... et al |
|--|

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.
4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

COMPUTER UPDATED

*MC ahz
3-1-02*

| | |
|--|--|
| Name and mailing address of the IPEA/ European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 | Authorized officer Senkel, H Tel. +49 89 2399-8071 |
|--|--|



PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

| | | |
|---|---|---|
| Applicant's or agent's file reference 31978-165733 | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
| International application No. PCT/US00/25833 | International filing date (<i>day/month/year</i>) 21/09/2000 | Priority date (<i>day/month/year</i>) 21/09/1999 |
| International Patent Classification (IPC) or national classification and IPC A61K51/04 | | |
| <p>Applicant THE GOVERNMENT OF THE USA, asrepresented... et al</p> | | |
| <p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheets.</p> | | |
| <p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input type="checkbox"/> Certain observations on the international application | | |

| | |
|---|--|
| Date of submission of the demand 07/03/2001 | Date of completion of this report 25.02.2002 |
| Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 | Authorized officer Beeck, M Telephone No. +49 89 2399 8473 |



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US00/25833

I. Basis of the report

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):
Description, pages:

1-27 as originally filed

Claims, No.:

1-40 as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
 - the language of publication of the international application (under Rule 48.3(b)).
 - the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).
3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.:
- the drawings, sheets:

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/25833

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:
- the entire international application.
 - claims Nos. 1-19.

because:

- the said international application, or the said claims Nos. 1-19 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet
 - the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
 - the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
 - no international search report has been established for the said claims Nos. .
2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
- the written form has not been furnished or does not comply with the standard.
 - the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | |
|---------------------|------|--------------|
| Novelty (N) | Yes: | Claims 8-40 |
| | No: | Claims 1-7 |
| Inventive step (IS) | Yes: | Claims 20-40 |
| | No: | Claims 1-19 |

Industrial applicability (IA) Yes: Claims 20-40

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US00/25833

No: Claims

2. Citations and explanations
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US00/25833

SECTION III:

Claims 1 to 19 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the **industrial applicability** of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

SECTION V:

- 1) The documents are numbered according to their sequence in the search report.
- 2) The examination has been carried out assuming that the priority is valid, so that P-document D1 has not been taken into consideration.
- 3) Document D2 describes a study with patients wherein PET is used for measuring the accumulation of ¹⁸F-tamoxifen in cardiac tissue (see the abstract).

Therefore the subject-matter of claims 1 to 7 is not novel (Article 33 (2) PCT).

- 4) The subject-matter of claims 8 to 12 differs from document D2 in that a modulator is added.

However, as long as the modulator is not further specified, the subject-matter of these claims is obvious for the person skilled in the art.

The same applies to the subject-matter of claims 13 to 19, where a further antitumour drug is added, which is obvious for the person skilled in the art.

Therefore the subject-matter of claims 8 to 19 does not involve an inventive step (Article 33 (3) PCT).

- 5) The subject-matter of claims 20 to 40 differs from the above cited prior art in that the anticancer compounds are other than tamoxifen and in that they are labelled with ¹¹C instead of with ¹⁸F.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US00/25833

In view of these differences the subject-matter of claims 20 to 40 is not obvious for the person skilled in the art, so that it involves an inventive step.

- 6) For the assessment of the present claims 1 to 19 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| | | |
|--|---|--|
| Applicant's or agent's file reference 31978-165733 | FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. | |
| International application No. PCT/US 00/ 25833 | International filing date (day/month/year) 21/09/2000 | (Earliest) Priority Date (day/month/year) 21/09/1999 |

Applicant

THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
 - contained in the international application in written form.
 - filed together with the international application in computer readable form.
 - furnished subsequently to this Authority in written form.
 - furnished subsequently to this Authority in computer readable form.
 - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. Certain claims were found unsearchable (See Box I).

3. Unity of invention is lacking (see Box II).

4. With regard to the title,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

5. With regard to the abstract,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/25833

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K51/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EMBASE, BIOSIS, EPO-Internal, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X, P | LEVCHENKO A ET AL: "Evaluation of 11C-colchicine for PET imaging of multiple drug resistance." JOURNAL OF NUCLEAR MEDICINE, (2000 MAR) 41 (3) 493-501., XP002175044 abstract --- TOMIO INOUE ET AL.: "Preliminary study of cardiac accumulation of F-18 Fluorotamoxifen in patients with breast cancer." CLINICAL IMAGING, vol. 21, 1997, pages 332-336, XP001015520 abstract --- | 1-19 |
| X | --- | 1-19 |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

12 September 2001

Date of mailing of the international search report

25/09/2001

Name and mailing address of the ISA
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Fax (+31-70) 340-3016

Authorized officer

Berte, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/25833

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|---|-----------------------|
| Y | SHIELDS A.F. ET AL: "Carbon - 11 -thymidine and FDG to measure therapy response." JOURNAL OF NUCLEAR MEDICINE, (1998) 39/10 (1757-1762). , XP002175045 abstract | 1-40 |
| X | KUH, HYO-JEONG ET AL: "Determinants of paclitaxel penetration and accumulation in human solid tumor" J. PHARMACOL. EXP. THER. (1999), 290(2), 871-880 , XP001015558 abstract | 1-31 |
| Y | SCHIRBEL, ANDREAS: "Synthesis of n. c.a. PET -radiotracers with carbon - 11" BER. FORSCHUNGSZENT. JUELICH (1998), JUEL-3602, 1-110 PP. , XP002175046 | 1-40 |
| X | the whole document | 1 |
| X | KANGAS, LAURI ET AL: "Biodistribution and scintigraphy of 11C-toremifene in rats bearing DMBA-induced mammary carcinoma" PHARMACOL. TOXICOL. (COPENHAGEN) (1989), 64(4), 373-7 , XP001015546 abstract; figures 1,3; tables 1,2 | 1-19 |
| X | HENDRIKSE NH. ET AL.: "a new in vivo method to study P-glycoprotein transport in tumors and the blood barrier." CANCER RESEARCH, vol. 15, no. 59, May 1999 (1999-05), pages 2411-2416, XP002175047 MD US cited in the application abstract | 1-19, 32-38 |
| X | WO 92 19264 A (UNIV NEW MEXICO) 12 November 1992 (1992-11-12) abstract; claims | 1,8-12 |
| A | JOSIEN, HUBERT ET AL: "A general synthetic approach to the (20S)-camptothecin family of antitumor agents by a regiocontrolled cascade radical cyclization of aryl isonitriles" CHEM.--EUR. J. (1998), 4(1), 67-83 , XP001015572 abstract; figures | 1,39,40 |
| | | -/- |

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/25833

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | <p>RAO CH.S. ET AL: "Synthesis and evaluation of '14C!-labelled and fluorescent-tagged paclitaxel derivatives as new biological probes." BIOORGANIC AND MEDICINAL CHEMISTRY, (1998) 6/11 (2193-2204). , XP001015574</p> <p>page 2194, column 1, paragraph 1 page 2196, column 2, paragraph 2 -page 2197, column 1, paragraph 1 page 2200, column 1, paragraph 1</p> <p>---</p> | 1-27 |
| X | <p>PENCO S ET AL: "SYNTHESIS OF 14 CARBON-14 DAUNORUBICIN AND DOXORUBICIN." J ANTIBIOT (TOKYO), (1977) 30 (9), 773-774. , XP001015786</p> <p>figure 1</p> <p>-----</p> | 1-19, 32-38 |

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-19 relate to an extremely large number of possible compounds. In fact, the claims contain so many options that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely the compounds and closely related homologous compounds mentioned in the other claims and the examples.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/25833

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

Although claim(s) 1-19 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

National Application No

PCT/US 00/25833

| Patent document cited in search report | Publication date | | Patent family member(s) | Publication date |
|--|------------------|----|-------------------------|------------------|
| WO 9219264 | A 12-11-1992 | US | 5240693 A | 31-08-1993 |
| | | US | 5401489 A | 28-03-1995 |
| | | WO | 9219264 A1 | 12-11-1992 |
| | | US | 6033644 A | 07-03-2000 |
| | | US | 5906807 A | 25-05-1999 |

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



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29 March 2001 (29.03.2001)

PCT

(10) International Publication Number
WO 01/21219 A2

(51) International Patent Classification⁷: **A61K 51/04**

Lawrence [US/US]; 12202 Judson Road, Wheaton, MD 20902 (US).

(21) International Application Number: **PCT/US00/25833**

(74) Agent: **SCHNELLER, Marina; Venable, P.O. Box 34385, Washington, DC 20043-9998 (US).**

(22) International Filing Date:
21 September 2000 (21.09.2000)

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(25) Filing Language: **English**

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(26) Publication Language: **English**

Published:

— *Without international search report and to be republished upon receipt of that report.*

(71) Applicant (*for all designated States except US*): **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY OF HEALTH AND HUMAN SERVICES [US/US]; Office of Technology Transfer, National Institute of Health, 6011 Executive Boulevard, Suite 325, Rockville, MD 20852 (US).**

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **COLLINS, Jerry, M. [US/US]; 1512 Auburn Avenue, Rockville, MD 20850 (US). KLECKER, Raymond, N. [US/US]; 1420 Colesberg Street, Silver Spring, MD 20905 (US). ANDERSON,**

WO 01/21219 A2

(54) Title: **IMAGING OF DRUG ACCUMULATION AS A GUIDE TO ANTITUMOR THERAPY**

(57) **Abstract:** The present invention describes the use of radio-labeled antitumor drugs in the treatment of solid tumors by the method of administering a radio-labelled anticancer drug to a patient and imaging at least a part of the patient using Positron Emission Tomography imaging. The method is used to monitor delivery of antitumor drugs to tumors and may be used to predict the effectiveness of therapy with a particular antitumor drug or combination of antitumor drugs, to assess the effectiveness of modulators of cellular accumulation, to individualize therapy and to evaluate the effectiveness of antitumor drugs with respect to particular cancers. Particularly preferred drugs are labeled taxanes, e.g., ¹¹C-paclitaxel and ¹¹C-docetaxel, labeled anthracyclines, e.g., ¹¹C-doxorubicin and ¹¹C-epirubicin, and other radio-labeled drug, e.g. ¹¹C-topotecan and ¹¹C-mitoxantrone. The invention further describes antitumor drugs labeled with the radioactive label ¹¹C and methods of preparing radio-labeled drugs.

IMAGING OF DRUG ACCUMULATION AS A GUIDE TO ANTITUMOR THERAPY

FIELD OF THE INVENTION

The present invention relates to the use of radio-labeled antitumor drugs in the diagnosis and treatment of cancers characterized by solid tumors. The invention also relates to radio-labeled antitumor drugs and their preparation.

BACKGROUND

Cancer treatment has made great progress in recent years. Many new therapies are becoming available, and many more patients are treated. Cancer is characterized by unrestricted cell growth; many cancer therapies work by inhibiting cell division. Since normal cells do not divide after maturation, inhibition of cell division primarily affects the cancer cells and this has been a focus of drug development. However, other cells are also affected by antitumor drugs to different degrees and cancer therapies are often extremely toxic to patients. There is also great variability in the efficacy of treatments. Some drugs are more effective than others for certain patients, for certain cancers, or at certain stages of treatment. Often, combinations of drugs with varying dosages are necessary for efficacious treatment, requiring considerable experimentation to optimize drugs and doses.

Among the agents affecting the therapeutic benefits of cancer drugs are the multi-drug resistance transporter (MDR), also called p-glycoprotein (PGP) or ABCB1. It has recently become clear that MDR is one of a family of such transporters. Klein I, Sarkadi B, Varadi A. An inventory of the human ABC proteins. *Biochim. Biophys. Acta* 1999; 1461:237-62. The expression of these proteins can be highly variable. MDR is not expressed in all cancer cells, and may be present at variable levels. The expression of MDR affects drug efficacy by altering drug accumulation at the tumor. Because of the variable efficacy of antitumor drugs caused by this and other factors, a critical part of therapeutic monitoring involves determining the drug location in the body, its half-life, and the range of mechanisms that limit its effectiveness.

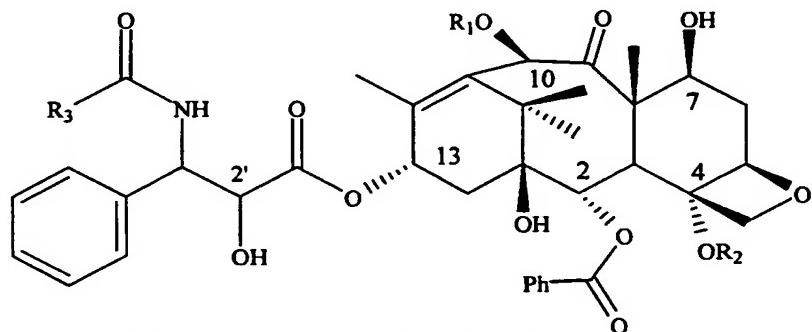
Accumulation of a drug reflects the net balance over time of influx (delivery to the tumor) and efflux (removal from the tumor). Influx and efflux are equally important, but recent research has focused upon a series of transport proteins that function as efflux pumps for taxanes, anthracyclines, and other drugs. There are many reasons why a tumor may not

be sensitive to a particular drug. However, the first parameter to evaluate is accumulation of drug by the tumor. If the drug doesn't accumulate in the tumor, there won't be an effect. Adequate accumulation is always necessary for drug activity. Thus, a method to determine accumulation of a drug in the tumor could be the first step in therapeutic decision-making for 5 the drug.

Traditional approaches to the determination of drug uptake and retention (drug accumulation in the tumor) have been invasive and most frequently require obtaining a biopsy from the patient. In addition to the discomfort and risks associated with biopsy procedures, only a small sample of tissue is typically obtained, which may not be representative of the 10 entire region.

Taxanes

One class of drugs which has proved particularly useful in the treatment of cancer, including solid tumors such as breast cancer, has been the taxanes. Taxanes are diterpenoid compounds with a complex taxane ring as the nucleus. The taxane paclitaxel (I) (Taxol ®) 15 was initially isolated from yew bark, although the compound may now be prepared synthetically. A modification of the side chains of paclitaxel has yielded another clinically effective compound, docetaxel (II). Other taxanes have also been developed, and are at various stages of preclinical and early clinical testing. These analogs include differences in functional groups attached to the main baccatin nucleus, as well as different side chains 20 attached at the C-13 position.



(I) Paclitaxel: R₁ = R₂ = acetate, R₃ = Ph

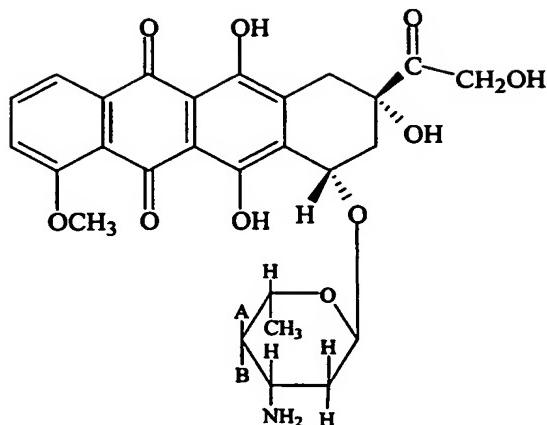
(II) Docetaxel: R₁ = OH, R₂ = acetate, R₃ = OC(CH₃)₃

Taxanes, like the vinca alkaloids and colchicine, work by interfering with microtubules, thereby inhibiting mitosis. Taxanes antagonize disassembly of microtubules, by promoting tubulin polymerization, inducing microtubule bundles to form. This leads to arrest of mitosis, and ultimately to cell death. The rate of cell death is proportional to 5 concentration of drug and length of time of administration. Taxanes are highly insoluble and are commonly administered in a solution of surfactants and other vehicles such as ethanol. (Hardman, J.G. and Limbird, L.E.,(eds) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, Chapter 51, McGraw-Hill, New York, 1996)

The taxane class of anticancer drugs has demonstrated remarkable activity. Docetaxel 10 and paclitaxel, the first two approved drugs in this class, have already altered the standard treatments for breast, lung, and ovarian tumors. Crown J, O'Leary M. The taxanes: an update. *Lancet* 2000; 355(9210):1176-8. Burris HA 3rd. Docetaxel (Taxotere) in the treatment of cancer. *Semin. Oncol.* 2000; 27 (2 Suppl 3):1-2. The full scope of antitumor activity for docetaxel and paclitaxel is still under active investigation, with new uses emerging for other 15 tumor types. For example, at present there is considerable interest in the use of docetaxel for treatment of prostate cancer. Oh WK, et al. Docetaxel (Taxotere)-based chemotherapy for hormone-refractory and locally advanced prostate cancer. *Semin. Oncol.* 1999; 26 (5 Suppl 17):49-54; Petrylak DP. Docetaxel (Taxotere) in hormone-refractory prostate cancer. *Semin. Oncol.* 2000; 27 (2 Suppl 3):24-9. Other molecules in the taxane class are at much earlier 20 stages of clinical testing.

Anthracyclines

Anthracyclines represent another important class of antitumor drugs. Representative anthracycline drugs include doxorubicin (III) and epirubicin (IV). These anthracyclines are leading agents for the treatment of many tumors, notably breast cancer, lung cancer, and 25 sarcomas. Doroshow JH. Anthracyclines and anthracenediones. In: Chabner BA and Longo DL, *Cancer Chemotherapy and Biotherapy*, 2nd Edition, p. 409, Lippincott-Raven, Philadelphia, 1996. Several mechanisms of action have been proposed, based upon the intercalation of the anthracycline molecules with DNA, and subsequent disruptions of cellular functioning.

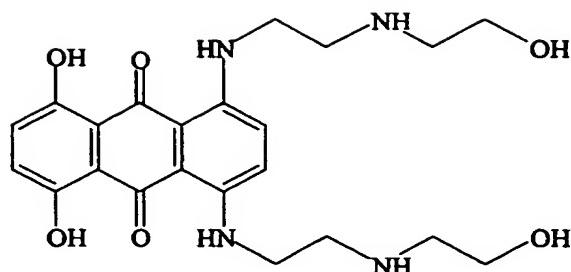


(III) Doxorubicin: A=H; B=OH

(IV) Epirubicin: A=OH; B=H

Other Antitumor Drugs

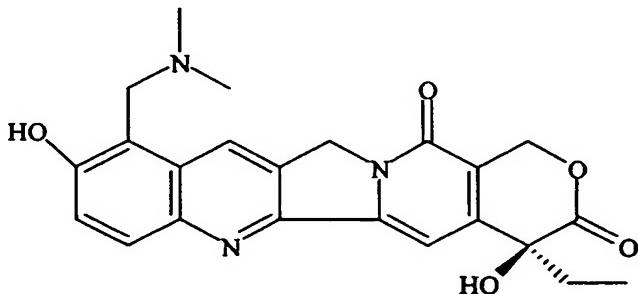
Because the discovery of anticancer drugs has been well-funded over the last half-century, drugs from a variety of chemical classes are now in routine clinical use. Mitoxantone (V) is an anthracenedione, a chemical class closely related to anthracyclines. Mitoxantrone was originally developed in an attempt to replace anthracyclines because the anthracenediones have lower cardiac toxicity. However, the antitumor activity of mitoxantrone was generally disappointing and it has a relatively narrow niche in clinical use. Recently, however, mitoxantrone has been demonstrated to substantially reduce the severe pain associated with metastatic prostate cancer, and has changed the management of this large group of cancer patients. Tannock IF, Osoba D, Stockler MR, Ernst DS, Neville AJ, Moore MJ, Armitage GR, Wilson JJ, Venner PM, Coppin CM, Murphy KC. Chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant prostate cancer: a Canadian randomized trial with palliative end points. *J. Clin. Oncol.* 1996; 14(6):1756-64.



(V) Mitoxantrone

Camptothecin is a natural product found in the bark and wood of a Chinese tree.

- 5 Although it was found to be too difficult for clinical use itself, a number of derivatives have demonstrated clinical activity. Takimoto CH, Arbuck SG. The Camptothecins. In: Chabner BA and Longo DL, *Cancer Chemotherapy and Biotherapy*, 2nd Edition, p. 463, Lippincott-Raven, Philadelphia, 1996. This class of drugs appears to work by inhibiting the action of topoisomerase I, a key enzyme for the integrity of DNA structure. Irinotecan (CPT-11) was
10 initially approved for treatment of colorectal cancer, and topotecan (VI) initially approved for



(VI) Topotecan

- ovarian cancer. Both of these two drugs and other camptothecin analogs are acquiring new uses as testing continues. Topotecan has shown activity in lung cancer, and is now also approved for treating these patients. In addition, topotecan is particularly important from the perspective of PET imaging because it is direct-acting (irinotecan is a prodrug that must be
15 activated), and also has limited catabolism, so that the parent molecule is the principal circulating species .

Determination of Sensitivity or Resistance

Although drugs having excellent antitumor activity e.g. taxanes, anthracyclines and others, have been identified, not all tumors respond to a given therapy. Furthermore, all patients are exposed to the risk of severe, life-threatening toxicity with these drugs whether 5 or not their tumors respond. An important tool for individualizing therapy would be a method to rapidly determine whether a specific tumor will be likely to respond to a particular drug without exposing the patient to toxic levels of the drug.

In addition to avoiding needless toxicity, a rapid determination is also important because of the tendency of tumors to become more difficult to treat with time. Thus, if one 10 treatment can be predicted to be unsuitable, alternative treatments can be explored without waiting months to determine that the first treatment did not work.

In some cases, the cause of treatment failure can not be determined. In other cases, a specific biochemical or molecular mechanism can be ascertained. For example, the tumor 15 cells may be intrinsically sensitive to the drug, but inadequate amounts of drug are accumulated in the tumor. There are multiple reasons for drug accumulation failures, but, regardless of the underlying cause, the empirical demonstration that adequate (or inadequate) drug was accumulated has enormous medical value in terms of treatment selection for individual patients. Determining drug accumulation levels in a specific tumor pre-treatment would thus provide a great value in exploring a wide range of treatment options.

20 In addition to the benefits for individual patients, determination of drug delivery has benefits for the general patient population and for the process of drug development. General patient populations include a mixture of tumors which are chemosensitive and chemoresistant. The demonstration of a substantial effect is made difficult when the responding tumors are diluted in a pool of nonresponding tumors. Thus, any technique which can find tumors likely 25 to respond before treatment has begun will produce an enriched study population, and greatly decrease the numbers of patients required to test the overall potential benefit of the drug.

Position Emission Tomography (PET) imaging or scanning uses positron emitter labeled tracers. Positrons are positively charged electrons which result from the decay of a proton rich and neutron deficient isotope. These emitters are generally short lived. Most 30 positron emitters are produced in medical cyclotrons or accelerators. The half life of ¹¹C is 20 min and of ¹⁸F is 110 minutes. PET cameras have a spatial resolution of several millimeters and can be used to image the entire body.

The concept of trying to find radiolabeled probes that would be ideal for the investigation of one or more transport pumps should not be confused with the concept of measuring accumulation of the specific drug to guide therapy. The literature is filled with research that attempts to define drug transport systems. Many groups are attempting to find 5 the "ideal" probe for each transporter. The emerging problem is an explosion in the numbers of transporters that are being discovered. Thus, attempts to guide therapy with a drug based upon ideal probes is fraught with difficulty and confounded by the multiplicity of transport mechanisms, which will vary from tumor-to-tumor.

Clinical attempts to measure drug accumulation with imaging have been reported, 10 including the use of ^{11}C -verapamil, ^{11}C -daunorubicin, or $^{99\text{m}}\text{Tc}$ -sesta-MIBI. All have been limited because they only target MDR and have additional difficulties.

Verapamil is known to interact with the MDR efflux pump, and Hendrikse *et al.* have demonstrated that images can be obtained with ^{11}C -verapamil in rats. Hendrikse NH, de Vries EG, Eriks-Fluks L, van der Graaf WT, Hospers GA, Willemse AT, Vaalburg W, Franssen 15 EJ. A new *in vivo* method to study P-glycoprotein transport in tumors and the blood-brain barrier. *Cancer Res.* 1999; 59:2411-6. Their work also showed that modulation of MDR *in vivo* could be demonstrated in rats with ^{11}C -verapamil. Although ^{11}C -verapamil may be an elegant probe for MDR *per se*, it is neither structurally nor functionally related to the taxanes, anthracyclines, anthracenediones, camptothecin analogs, or any other approved anticancer 20 drugs. It is also important to recognize that, in humans, there is rapid and extensive catabolism of verapamil. Schomerus M, Spiegelhalder B, Stieren B, Eichelbaum M. Physiological disposition of verapamil in man. *Cardiovasc. Res.* 1976; 10(5):605-12. Verapamil itself constitutes only a small fraction of the circulating radioactivity, so the interpretation of the total radioactivity signal obtained with PET is problematic.

Also, although daunorubicin has been used as a probe in cell culture, where it is a stable molecule, it is not a stable molecule in the body and imaging with this component is not very useful. In humans, daunorubicin is converted rapidly by carbonyl reductase to its alcohol metabolite, daunorubicinol. Thus, the signal observed via external PET imaging of labeled daunorubicin is a mixture of these two chemical entities, which can complicate the 25 interpretation. The plasma ratio of metabolite-to-parent is about 2.5:1 (Galettis P, Boutagy J, Ma DD. Daunorubicin pharmacokinetics and the correlation with P-glycoprotein and response in patients with acute leukaemia. *Br. J. Cancer* 1994; 70(2):324-9), so it is possible 30

that the metabolite is the species primarily being imaged. However, it is also possible that tissue uptake is more favorable for the parent, so a different ratio might be found.

5 ^{99m}Tc-Sestamibi, which is routinely used for cardiac imaging, has also been explored for tumor imaging. As in the case for verapamil, neither the structural nor functional properties of sestamibi resemble those for anticancer drugs. The clinical imaging results are mixed. This probe seems to have the ability to detect tumors and monitor response in some clinical settings. Mankoff DA, Dunnwald LK, Gralow JR, et al. Monitoring the response of patients with locally advanced breast carcinoma to neoadjuvant chemotherapy using ^{99m}Tc -sestamibi scintimammography. *Cancer* 1999; 85(11):2410-23. It is reported that the efflux rate of ^{99m}Tc -sestamibi correlates with antitumor response, at least for one stage of breast cancer. Ciarmiello A, Del Vecchio S, Silvestro P, et al. Tumor clearance of technetium 99m-sestamibi as a predictor of response to neoadjuvant chemotherapy for locally advanced breast cancer. *J. Clin. Oncol.* 1998; 16:1677-83. However, in lung cancer, no predictive value was found. Sasaki M, Kuabara Y, Yoshida T, et al. Can 99m-Tc-mibi-SPECT predict 10 the treatment response of lung cancer? *J. Nucl. Med.* 2000; 41:286P.

15

The invention provides advantages that were not previously available by providing a non-invasive method for determining potential drug efficacy by measuring actual drug accumulation in tumors. This invention allows for the efficient determination of the potentially most efficacious treatment plan for antitumor therapy by allowing for 20 individualized optimization of drugs and dosage.

The invention offers the additional, previously unrealized advantage of developing individualized antitumor therapies specific to a particular patient with a particular type of tumor in a particular stage of development.

25 The invention also provides a method for determining the effectiveness of particular drugs as treatment for particular cancers over a broad range of the population.

SUMMARY OF THE INVENTION

Non-invasive, external imaging methods to visualize the location of a drug in the body avoid the need for biopsies and also have the capability of scanning large areas of the body, indeed, the entire body if necessary.

30 Since a drug is only effective if it reaches the desired site of action, a method to determine the location of the drug after administration allows monitoring of the potential

effectiveness of drug administration. Antitumor drugs in particular are often toxic, and monitoring could reduce the time required to find the most effective and targeted therapy. Administration of a radio-labeled antitumor drug (preferably, the chemotherapeutic drug itself) which has been radio-labeled, preferably with a positron emitter, can provide external 5 monitoring of drug accumulation in the tumor and/or normal host tissue by use of imaging technologies, preferably with a PET scanner.

The present invention describes a method of labeling antitumor drugs with positron emitters, preferably the radioactive label ^{11}C . The invention further describes the use of labeled antitumor drugs in the treatment of solid tumors. The invention is also a method of 10 using labeled antitumor drugs to monitor the accumulation of drugs to solid tumors and to monitor modulators of cellular accumulation of drugs which act on mechanisms in the cancer 15 cells of the tumors that prevent uptake and retention of drugs.

In one aspect, the invention is a method of measuring the accumulation of antitumor drugs by solid tumors comprising, administering an antitumor drug labeled with a positron-emitter to a patient having a solid tumor, and imaging at least part of the patient using PET. 15 Typically, solid tumors include breast, lung, ovarian, gastrointestinal, prostate, sarcoma and head and neck tumors. The measurement of accumulation of the drug may be measured either before or during a particular treatment regimen, and may be used to determine the efficacy of 20 an antitumor drug for treating solid tumors; to measure the effectiveness of modulators of cellular accumulation including modulators of efflux and influx mechanisms; to measure the effectiveness of a combination of antitumor drugs where one or more of the drugs is labeled 25 and the drugs are administered either simultaneously or sequentially; and for any other purpose for which measuring cellular accumulation of an antitumor would be beneficial. Preferred drugs for use in the invention include ^{11}C -paclitaxel, ^{11}C -docetaxel, ^{11}C -doxorubicin, ^{11}C -epirubicin, ^{11}C -mitoxantrone, and ^{11}C -topotecan, although any drug for the 25 treatment of solid tumors in radio-labeled form may be used.

In another aspect, the invention is a composition which is a radio-labeled drug. In particular, radio-labeled taxanes, especially ^{11}C -paclitaxel and ^{11}C -docetaxel; radio-labeled anthracyclines, especially ^{11}C -doxorubicin and ^{11}C -epirubicin and other radio-labeled 30 antitumor drugs, especially ^{11}C -topotecan. The invention is also a method of preparing radio-labeled drug. In particular, radio-labeled taxanes, especially ^{11}C -paclitaxel and ^{11}C -docetaxel; radio-labeled anthracyclines, especially ^{11}C -doxorubicin and ^{11}C -epirubicin and other radio-

labeled antitumor drugs, especially ^{11}C -topotecan.

The scope of this invention covers the preparation and diagnostic uses of antitumor drugs including paclitaxel, docetaxel, doxorubicin, epirubicin and others labeled with a positron emitter, preferably ^{11}C . Based upon the images obtained, the likelihood of success
5 for treatment of a patient's tumor with antitumor drugs can be predicted, as well as the utility of any modulators of drug delivery.

One goal of the present invention is to avoid exposing patients to toxic drugs that have no potential for benefit. The screening procedure of the present invention allows a rapid determination of whether a given tumor will be likely to respond to a particular drug. Such
10 a rapid determination is important for other reasons, including the tendency of tumors to become refractory with time. Thus, immediate imaging with ^{11}C -paclitaxel, ^{11}C -docetaxel, ^{11}C -doxorubicin, ^{11}C -topotecan, ^{11}C -mitoxantrone or other radio-labeled antitumor drugs has the advantage of selecting patients with tumors that are likely to respond to therapy with the particular drug that is used. Further, the impact of various doses and schedules for delivery
15 can be monitored *in situ* at the actual tumor under treatment conditions. This is important since levels of MDR and other modifiers of drug efficacy are often induced or altered during the course of therapy. It is within the scope of the present invention to re-test patients as often as therapeutically or diagnostically necessary to assess the changing efficacy of treatment.

The method of the present invention involves administration of tracer, nontoxic
20 quantities of an antitumor drug that has been radio-labeled with a positron emitter. Imaging technologies such as a PET (Positron Emission Tomograph) scanner, can provide the ability to externally monitor drug delivery to the tumor and/or normal host tissue. It is particularly noteworthy that this procedure is noninvasive and can be used in a prognostic sense, i.e., before a particular drug is administered in therapeutic quantities.

25 Preferred drugs for use in the invention are docetaxel, paclitaxel, doxorubicin, epirubicin, mitoxantrone and topotecan that have been radio-labeled with a positron emitter. Although a wide range of labeling techniques are known, for example replacing H with ^{18}F , the radiolabelled drug is preferably identical to the non-labeled drug except that it contains a radio-labeled atom. Thus, preferred radio-labeled drugs are those in which a naturally
30 occurring atom is replaced with the same atom. Most preferably, ^{12}C is replaced with ^{11}C .

Others have used radio-labeled drugs to explore drug distribution in the body, but the present invention is specifically useful in measuring drug accumulation in a tumor in order

to make therapeutic decisions. For example, accumulation of a drug in a tumor may be measured to make predictions regarding effectiveness of the drug in a particular patient or against a particular tumor. Similarly, accumulation of an antitumor drug may be measured when the drug is co-administered with another antitumor drug or with a cellular accumulation modifier. Significantly, the present invention measures drug accumulation regardless of the mechanism of drug delivery and regardless of any particular transport pump (influx or efflux) that may be operating.

Further objectives and advantages will become apparent from a consideration of the description and examples.

10 DETAILED DESCRIPTION OF THE INVENTION

In describing preferred embodiments of the present invention, specific terminology is employed for the sake of clarity. However, the invention is not intended to be limited to the specific terminology so selected. All references cited herein are incorporated by reference as if each had been individually incorporated.

15 The term "a" is intended to mean at least one unless the context indicates otherwise.

Imaging Method According to the Invention

The present invention describes a method of visualizing antitumor drugs in the body. The drugs are first labeled by inserting a positron emitting isotope in the drug and 20 administered the drug to a patient. Preferably, the drug is labeled by replacing a normally occurring atom with a radioactive atom (e.g., ^{11}C for ^{12}C or ^{18}F for ^{19}F) in the drug structure. The antitumor drugs labeled with positron-emitting isotopes are then visualized by means of PET scan of the host's body. For the purpose of the present invention, a host includes any animal, preferably a human, to whom the positron emitting drugs of the present invention are 25 administered for any therapeutic, diagnostic, prognostic, or experimental purpose.

The object of labeling antitumor drugs is to determine and monitor the location of the drug in the body by imaging. For example, for taxane-type drugs, the invention may utilize ^{11}C -paclitaxel or ^{11}C -docetaxel. When cells accumulate antitumor drugs labeled with a positron-emitting isotope, the location of tissues comprising the cells can be determined by 30 a PET scan.

According to the invention, a positron-labeled anti tumor drug can be administered simultaneously with the non-radio-labeled antitumor drug to customize treatment under the actual conditions of use. Positron-labeled drugs may also be administered in test quantities to determine the likelihood that the drug would be an effective therapeutic.

5 Significantly, many antitumor drug metabolites are rapidly excreted in the bile, so that circulating radioactivity would be primarily the parent molecule.

Need for Exact Match of Drug and Imaging Profile

Within the limits of our present understanding of antitumor drug pharmacology, only the radio-labeled versions of the drugs themselves can be relied upon to predict accurately 10 therapeutic drug delivery. The use of any other molecule, no matter how apparently similar, potentially compromises the quality of the whole concept.

Recently, Li *et al.* have attempted to label paclitaxel with indium. Li C, Yu DF, Inoue T, Yang DJ, et al. Synthesis, biodistribution and imaging properties of indium-111-DTPA-paclitaxel in mice bearing mammary tumors. *J. Nucl. Med.* 1997;38:1042-7. These 15 investigators used Diethylenetriaminepentaacetic acid anhydride (DTPA), a chleating agent, to attach ¹¹¹In to paclitaxel. The distribution in the body was found to be significantly different for the ¹¹¹In-DTPA-paclitaxel than for the parent paclitaxel and was particularly affected by renal excretion. Because of these differences, it would require significant experimentation to characterize and correlate the properties of indium-DTPA-paclitaxel with 20 paclitaxel.

In addition to the above concerns, both ¹¹¹In-DTPA and ^{99m}Tc are inferior atoms for imaging compared with positron-emitters such as ¹¹C and ¹⁸F. Unlike ¹¹¹In-DTPA and ^{99m}Tc, positron emitters ultimately generate two coincident photons, which permits the three-dimensional position within the body to be determined. The sensitivity of positron emitters 25 such as ¹¹C is considerably greater, which permits smaller tumors to be imaged.

In contrast to the substitution of non-radio-labeled atoms with radio-labeled atoms, attaching "extra" atoms to the drug, or changing some of the atoms in the drug, creates an unacceptable situation, in the absence of additional information. Using taxanes as an example, replacement of a hydrogen atom with longer-lived isotopes of fluorine or iodine has 30 been attempted.

Ojima et al. report considerable differences in tumor cell sensitivity when fluorine

analogs of docetaxel are evaluated, particularly at the 3'-C locus. Ojima I, Inoue T, Chakravarty S. Enantiopure fluorine-containing taxoids: potent anticancer agents and versatile probes for biomedical problems. *J. Fluorine Chem.* 1999; 97:3-10. Most importantly, the largest differences were observed for cell lines resistant to docetaxel. This
5 variation in responsiveness might lead to some promising leads for alternative drug discovery. However, it would be misleading if the fluorinated molecule was used as a probe for decision-making regarding treatment with the parent docetaxel, because a favorable outcome would be projected in situations in which the tumor was actually resistant to docetaxel.

An iodinated version of paclitaxel has recently been proposed as a probe. John CS,
10 Cole CE, Kiesewetter DO, Eckelman WC. Synthesis, characterization, in-vitro cell binding, and biodistribution of radioiodinated [125-I]-paclitaxel. *J. Nucl. Med.* 2000; 41:229P. Although limited biodistribution data were reported, no attempt was made to demonstrate that it was comparable to the parent paclitaxel molecule. In fact, the iodine was placed on the phenyl ring of the benzoyl group attached to the amino moiety at the 3'-C position of
15 paclitaxel. This is the same portion of the molecule which produced variations for fluorinated analogs of docetaxel. It's also the major site of structural difference for docetaxel versus paclitaxel: substituting a tert-butyl group for the phenyl moiety.

Thus, considerable caution is required for any such modification. The burden of evidence that such a change is acceptable must be very high, particularly since the imaging
20 information will be used to guide decisions about therapy of patients with life-threatening disease.

In addition, the short half-life of ^{11}C (20 minutes) is advantageous when compared to the 6 hour half-life for $^{99\text{m}}\text{Tc}$. Thus, multiple interventions may be conducted and probed in sequence during a single clinic day with ^{11}C labeled drug, whereas $^{99\text{m}}\text{Tc}$ labeled drug would
25 be limited to one procedure per day, due to carryover of radioactivity from prior dosing. The shorter half-life of ^{11}C also substantially reduces the biohazard, compared with $^{99\text{m}}\text{Tc}$, since most of the injected radio-activity would have left the patient before the patient left the clinic. Importantly, while expensive and extensive preclinical toxicology studies are usually required prior to human testing of a drug variant, none are required for positron-labeled version at a
30 tracer dose of a currently-marketed drug. Radiodosimetry studies, of course, are still required.

In the case of taxanes, only paclitaxel and docetaxel are currently approved for therapeutic use. Other taxanes are under development, and might be approved and marketed

in the future. If so, then these would also be candidates for PET imaging. Thus, nothing in this disclosure is intended to limit the application of these concepts solely to taxanes such as ^{11}C -docetaxel or ^{11}C -paclitaxel. Similarly, doxorubicin and epirubicin are the major anthracyclines for solid tumor therapy, but these concepts are not limited to ^{11}C -doxorubicin 5 or ^{11}C -epirubicin. It is obvious to one skilled in the art that other classes of antitumor drugs may also be used in practicing the invention.

Assessment of Therapeutic Effects of Drugs on Particular Tumor Types

In an anticancer drug development program, the general strategy is to find the tumor types most likely to respond to a particular antitumor drug. Direct demonstration of clinical 10 benefit is critical. Using typical methods, these definitive studies require hundreds or thousands of patients, which is resource-intensive and takes time. It takes time to determine the success or failure in a general tumor type, and thus, to determine whether to continue investing money in clinical trials.

The invention may be used to assess the likelihood of success in using a particular 15 antitumor drug as a treatment for a particular type of tumor. According to the invention, a radio-labeled antitumor drug may be administered to patients having a particular tumor type. The accumulation of the radio-labeled drug in the tumors can be measured using the method of the invention. Because analysis of drug accumulation in the tumor may be measured directly and results will not be dependent on measuring the long term response of patients, the 20 number of patients required to conduct studies predicting drug effectiveness and the amount of time required to analyze the results may both be markedly reduced. In addition, the effects of modulators of cellular accumulation of drugs and the effectiveness of co-administration of more than one antitumor drug (as described below) may be addressed in the context of the broader patient population.

25 Individualization of Therapy for Patients

Because there are multiple reasons for drug accumulation failures, a successful interventional strategy generally requires knowledge of the most important factor for each situation. However, administration of a drug labeled with a positron emitter allows for monitoring of the success of modulation to be determined non-invasively with external 30 imaging techniques, if the radio-labeled agent is the same as the drug itself (e.g., paclitaxel), regardless of the mechanism(s) of drug delivery failure. Accordingly, if a tumor is known or

suspected to be resistant to docetaxel, paclitaxel, doxorubicin, epirubicin, or another antitumor drug, imaging with a positron-labeled version of the drug can determine if the failure to respond is due to inadequate accumulation.

Recently, researchers have found that the MDR transporter is one of many transporters and other similar mechanisms, which prevent the accumulation of drugs in cells. For example, there are a large family of ATP-binding cassette (ABC) transporters, including MDR, MRP, and others. Expression of such transport mechanisms will vary from tumor-to-tumor. If accumulation of the drug is determined to be inadequate, possibly due to the presence of such systems, one course of action that is under intensive research is the search for strategies to improve drug accumulation in the tumor, potentially converting the situation from a failure to a treatment success.

Because it is known that the failure to accumulate drugs in the tumor in adequate amounts is a major cause for treatment failure, intensive research efforts are underway to improve drug delivery and/or retention. Modulators of cellular accumulation mechanisms are often discovered in the laboratory which increase the accumulation of antitumor drugs in tumor cells. The present invention allows rapid determination of the usefulness of such a modulator for specific patients with positron labeled antitumor drugs, by directly assessing whether drug accumulation is improved. Thus, the invention may be used to assess the impact of various modulators, doses, and schedules for accumulation *in situ* at the actual tumor to be treated.

Antitumor drugs have also been given intraperitoneally for treatment of ovarian carcinoma, with encouraging results. The ability to measure drug concentration at the tumor and/or normal tissue is critical to the evaluation of the success or failure of the therapy. Thus, the invention provides a means for directly assessing drug accumulation, a marked advantage over simply measuring plasma concentrations. Using the invention, it is thus possible to adjust therapy to fit a particular clinical situation. For example, the effect of drug uptake and retention modulators on drug delivery can be directly measured.

Assessment of Modulating Strategies

One of the major determinants of cellular accumulation is the operation of the efflux pumps. Many antitumor drugs may be effluxed from cells wherein MDR or other efflux pumps or transporters are highly expressed. The drugs are effluxed before they can

accumulate. For example, taxanes may be prevented by efflux pumps from reaching their target, microtubules, and therefore do not bind to the microtubules. Thus, the presence of efflux transporters can prevent drugs from therapeutic action in the cell. Efflux transporters will also prevent accumulation of the radio-labeled antitumor drugs. In cells with highly
5 expressed efflux mechanisms, antitumor drugs do not accumulate. The failure to accumulate antitumor drugs by tumor cells can indicate that high levels of efflux pumps are expressed in these cells and preventing therapeutic levels from being attained.

For commonly-used chemotherapy drugs such as taxanes, anthracyclines and vinca alkaloids, many tumor cells are resistant because they prevent accumulation of the drug into
10 the cell. A common mechanism for this failure to accumulate a drug is the efflux pump MDR. In order to address these difficulties, modulators of cellular accumulation mechanisms (modulators) may be administered with an antitumor drug to increase drug accumulation in the tumor. By monitoring changes in accumulation when a modulator is added, the effectiveness of the modulator may be determined.

15 Other accumulation systems, for example, influx pumps or transporters, also exist and accumulation is the balance between influx and efflux rates. One skilled in the art will appreciate that similar principles apply to both efflux and influx systems. Thus, the present invention may be applied to influx pumps as well.

A variety of attempts to inhibit MDR have entered clinical testing, including
20 dexverapamil, PSC833 from Novartis, LY335979 from Lilly, GG918 from Glaxo and VX-853 from Vertex. Some surfactants, such as the solvent Cremophor®, which is used in the intravenous formulation of Taxol®, have been reported to be modulators of paclitaxel uptake and retention by tumor cells. The present invention may be utilized to monitor the effectiveness of these and other cellular accumulation modulators, such as the modulation of
25 efflux mechanisms by any modulator including use of excipients used in formulations of drugs.

Current clinical investigations of MDR modulators have only measured plasma concentrations of the drug itself. Since the purpose of modulation is to selectively improve accumulation of the drug to the tumor, the plasma data are not definitive. Because paclitaxel
30 and docetaxel are substrates for MDR, studies directed towards clarifying uptake of these drugs by tumor cells would be clinically useful. Measurement of drug concentration in sequential biopsies of the tumor, with and without modulator, could provide more definitive

comparisons, but are not practical in a clinical situation. However, according to the invention, ¹¹C-paclitaxel or ¹¹C-docetaxel can be used to directly assess the success or failure of these MDR-modulating strategies at the tumor itself. Similarly, doxorubicin and epirubicin are substrates for multiple efflux pumps, so that determination of the accumulation of ¹¹C-doxorubicin or ¹¹C-epirubicin would be clinically useful. The method according to the present invention utilizes positron-labeled antitumor drugs to measure the efficacy of MDR modulators of cancer cell efflux, influx, or other accumulation mechanisms.

The invention also envisions that substances which moderate or reduce efflux mechanisms could be co-administered with the radio-labeled antitumor drugs. Thus, it would be possible to determine an optimal dose of such a moderator that would allow therapeutic levels of antitumor drugs to accumulate in cells. Since the current list of known efflux pump moderators is quite low, and many are only known to work in cell-culture and not necessarily *in vivo*, the invention encompasses the use of labeled antitumor drugs as a research tool to find and explore other efflux moderators.

In addition to MDR, modulating strategies can be directed at other targets, including some which might be directed at decreasing normal host tissue concentrations as a way of reducing patient toxicity, rather than increasing tumor concentration. Overall, the same goal is sought: to increase selective action of the drug at the tumor site under conditions tolerable to the patient.

The present invention is useful in determining the level of impact of efflux type pumps or transporters on drug accumulation and thereby whether antitumor drugs are likely to be effective in the tumor imaged. When a radio-labeled antitumor drug is administered to a patient with a tumor with low drug accumulation, the tumors cannot be imaged by a PET scan, indicating that the tumor is unlikely to be effectively treated by the antitumor drug.

25 Assessment of Combination Chemotherapy Strategies

Often, when antitumor drugs are used therapeutically, maximal effects are achieved by co-administering more than one drug, such as the combination of a taxane with doxorubicin. The invention uses labeled antitumor drugs as a tool to determine optimal combinations of drugs that are co-administered. The determination of which drugs to co-administer will depend on the clinical circumstances of the disease to be treated. The decision

of whether to co-administer a drug and which drug to use will depend on the clinical decision of the practitioner.

Although the combination of taxanes with doxorubicin is very promising, only plasma pharmacokinetics are currently accessible to assess therapeutic strategy Gianni, L. et al.

5 Human pharmacokinetic characterization and in vitro study of the interaction between doxorubicin and paclitaxel in patients with breast cancer. *J. Clin. Oncol.* 15:1906-12, 1997; Gianni, L et al. Paclitaxel by 3-hour infusion in combination with bolus doxorubicin in women with untreated metastatic breast cancer: high antitumor efficacy and cardiac effects in a dose- and sequence-finding study. *J. Clin. Oncol.* 13:2688-2699, 1995.) An analogous 10 set of clinical trials with docetaxel and various anthracyclines are underway. Sparano JA, O'Neill A, Schaefer PL, et al. Phase II trial of doxorubicin and docetaxel plus granulocyte colony-stimulating factor in metastatic breast cancer: Eastern Cooperative Oncology Group Study E1196. *J. Clin. Oncol.* 2000;18:2369-77. Assessing the accumulation of ¹¹C-taxane can play a major role in dose and sequence decisions for these trials.

15 The present invention contemplates co-administration of radio-labeled antitumor drugs to determine efficacy of such combinations. Thus, using the imaging method of the invention, the effect of co-administration of more than one antitumor drug on drug accumulation may be determined. This determination may be made by assessing the accumulation of one or both of the antitumor drugs which are co-administered. Accordingly, 20 one or both of the co-administered antitumor drugs is labeled with a radio isotope and the accumulation of the radio-labeled drug at the tumor is measured using PET techniques.

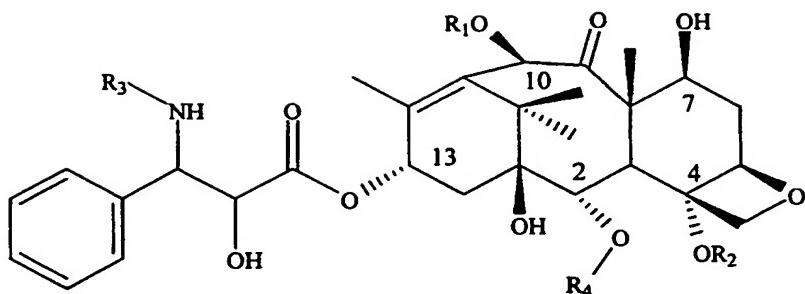
Synthesis of Positron Emitting Antitumor Drugs

In another aspect, the invention also relates to certain radio-labeled antitumor drugs useful in practicing the invention and methods of synthesizing radio-labeled drugs. In 25 particular, the invention is a method for synthesizing radio-labeled taxanes and anthracyclines.

The invention is not limited to the specific drugs and methods of the examples, but include any equivalents to those compositions and procedures as would be suggested to one of skill in the art.

Taxanes

30 In particular, the invention includes compounds having the formula:



where R₁ is selected from the group consisting of H, acetate and ¹¹C-acetate; R₂ is selected from the group of acetate and ¹¹C-acetate; R₃ is selected from the group consisting of benzoyl, ¹¹C-benzoyl and -CO₂C(CH₃)₃ and -¹¹CO₂C(CH₃)₃; R₄ is selected from the group consisting of benzoyl and ¹¹C-benzoyl, and wherein the compound contains at least one atom of ¹¹C.

5 Radio-labeled paclitaxel and docetaxel are preferred taxanes. Radio-labeled paclitaxel may have a ¹¹C labeled acetate in the 4-position or the 10-position, or may have a radio-labeled benzoyl group at the R₃ amide or at R₄ in the 2-position. Radio-labeled docetaxel may have a radio-labeled tert-butyl carboxyl group at R₃, acetate at R₂ or benzoyl at R₄. Typically, the

10 acyl groups are labeled at the carbonyl carbon.

The procedures for the synthesis of radio-labeled taxanes are modifications of those reported by Rao, KV et al. (*Synthesis and evaluation of some 10-mono- and 2',10-diesters of 10-deacetylpaclitaxel*. *J. Med. Chem.* 38:3411-14, 1990), and by Murray *et al.* in U.S. Patent No. 5,808,113. The present methods utilize different reagents and reaction conditions as compared to these reports. The differences improve the efficiency of the reaction with respect to addition of ¹¹C, i.e., via acetylation or benzoylation.

From the perspective of a large-scale manufacturing process, 10-deacetylpaclitaxel or the other taxane precursors are scarce and expensive resources, and the procedures are optimized for high yields of the taxane starting material. In this regard they are successful; e.g., Rao *et al.* report 85% conversion of 10-deactylpaclitaxel to paclitaxel. However, Rao *et al.* used approximately a 200-fold a molar excess of acetyl donor. Based upon acetyl groups, the yield of this reaction would be 0.5%, which is impractical for radio-synthesis of an ¹¹C-labeled PET probe. The present invention accomplishes the higher yields of 10-20% (based on acetyl donor) required to make PET imaging feasible. Thus, the present invention represents a major improvement over Rao *et al.* Using routine experimentation, a person

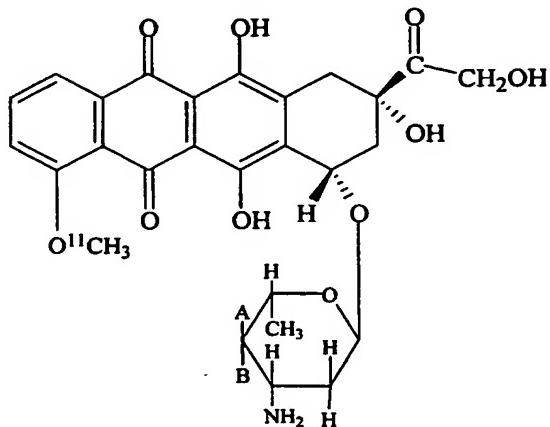
skilled in the art may make minor alterations in the synthesis as may be required to adapt the conditions to those routinely used at their particular radiotracer facility.

Verification of the identify of the product is obtained by comparison with authentic nonradioactive reference material of paclitaxel (available commercially, e.g., from Sigma 5 Chemical Company, Hauser Chemical Company, HandeTech Corporation) or docetaxel (available from Aventis/RPR). The final product, e.g., ^{11}C -paclitaxel or ^{11}C -docetaxel, is purified (e.g., using a solid-phase extraction cartridge) and prepared for intravenous injection in a suitable solvent (e.g., normal saline, Cremophor^R, ethanolic solution, Tween 80).

Similar methods may be useful for preparing other radio-labeled taxanes by incorporation ^{11}C into ester or carbamate substituents on the baccatin nucleus or side chains. 10

Anthracyclines

The invention also includes radio-labeled anthracyclines and particularly radio-labeled doxorubicin and epirubicin having the general formula:



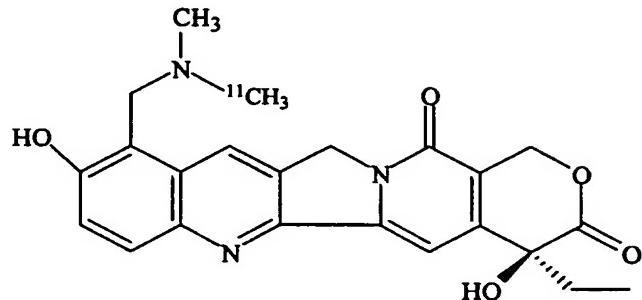
15 wherein one of A is H and B is OH (^{11}C -doxorubicin) or A is OH and B is H (^{11}C -epirubicin).

Due to the lack of commercial availability of a suitable anthracycline antitumor drug precursor for radiolabeling, several initial steps are required to generate a precursor for preparing radio-labeled doxorubicin and related drugs. In essence, after protecting the amine group in the sugar and the -OH groups, the methyl group at the 4-O-position is removed, 20 producing a suitable precursor. Because these steps are performed prior to radiolabeling, high yield and rapid reactions are not essential. Radiolabeling of the precursor can be accomplished with ^{11}C -methyl iodide, a standard radiolabeling reagent. Deprotection

produces the desired radio-labeled product.

Other Antitumor Drugs

The invention also includes other radio-labeled anti-tumor drugs and particularly radio-labeled topotecan having the general formula:



5 Radiolabeled topotecan may be prepared from N-desmethyl topotecan, which has been reported in the literature (Rosing H, Herben VMM, van Gortel-van Zomeren DM, et al, Isolation and structural confirmation of N-desmethyl topotecan, a metabolite of topotecan, *Cancer Chemotherapy and Pharmacology* (1997) 39:498-504), but is not commercially available. The N-desmethyl topotecan is prepared from commercial topotecan through a
10 procedure adapted from the procedure of Rao,et.al. (Rao,P.N., Acosta,C.K., Cessac,J.W., Bahr,M.L., Kim,H.K. Synthesis of N-desmethyl derivatives of 17 a-acetoxy-11b-(4-N,N-dimethylaminophenyl)-19-norgogna-4,9-diene-3,20-dione and mifepristone. *Steroids* 1999; 64:205-212. The ^{11}C -topotecan is prepared from N-desmethyl topotecan by methylation with ^{11}C -methyl iodide.

15 Another potentially useful radio-labeled drug for use in practicing the invention is ^{11}C -mitoxantrone. Current efforts for preparing this drug by placing a ^{11}C -label in one of the hydroxyethyl groups have not been successful.

EXAMPLES

Example 1: Synthesis of ¹¹C-paclitaxel

10-Deacetylpaclitaxel (6 mg) (commercially available) in pyridine (0.5 ml) was reacted with chlorotriethylsilane (0.1 ml) for 1 hour at 60°C to yield 7,2'-di-(triethylsilyl)-10-deacetylpaclitaxel. This molecule is the immediate precursor for radiolabeling; it is stable at room temperature and can be stored until needed.

To a solution of (50 μ g, 60 nmol) of 7,2'-di-(triethylsilyl)-10-deacetylpaclitaxel, was added dimethylaminopyridine solution (90 μ l of 300 mg/ml in methylene chloride), and of tert-butyl diphenyl chlorosilane (20 μ L). Acetyl chloride (10 μ l of a 1:5000 solution in methylene chloride, 25 nmol) was then added and the mixture heated to 105°C for 10 minutes. The silyl groups were removed within 2 minutes by adding of methylene chloride (400 μ l) and tetrabutylammonium fluoride solution (50 μ l of 1 M solution in tetrahydrofuran).

Verification of the identify of the product via HPLC and mass spectrometry was obtained by comparison with authentic reference material of paclitaxel (available commercially). The overall yield, based upon the acetyl donor, was 10-20%.

This procedure works with sufficient yield (10-20% overall), in the required time limit (15 minutes from addition of acetyl chloride until beginning of clean-up), and with small quantities of material (e.g., 50 μ g). Thus, ¹¹C-paclitaxel may be prepared simply by substituting ¹¹C-acetyl chloride for unlabeled acetyl chloride.

On the day of use, ¹¹C is prepared by a cyclotron in the form of ¹¹C-CO₂. This radio-labeled carbon dioxide is rapidly converted to ¹¹C-acetyl chloride using published procedures. Luthra SK, Pke VW, Brady F. Preparation of some NCA [1-¹¹C] acid chlorides as labelling agents. *Appl. Radiat. Isot.* 1990; 41:471-6. The final product, ¹¹C-paclitaxel, is purified (e.g., using a solid-phase extraction cartridge or HPLC) and prepared for intravenous injection in a suitable solvent (e.g., normal saline, Cremophor^R or ethanolic solutions).

Example 2: Alternative Synthesis of ¹¹C-paclitaxel

Benzoyl chloride (8 μ l of 1:1000 dilution in acetonitrile) is added to a solution of paclitaxel primary amine (50 μ g) (commercially-available) in acetonitrile (200 μ l). After 2 minutes at room temperature, 60% recovery of product was obtained.

Conversion of this procedure to radio-synthesis requires preparation of ¹¹C-benzoyl chloride, which is known in the art by following typical Gringard reaction schemes. Mathews

WB, Burns HD, Danals RF, Rabert HT, Naylor EM. Carbon-11 labeling of a potent, nonpeptide, AT1-selective angiotensin-II receptor antagonist, MK-996. *J. Labelled Compounds and Radiopharmaceuticals* 1995; XXXVI:729-37. For ¹¹C-paclitaxel, purification may be accomplished by HPLC or by adding acidic water and methylene chloride.

5 Example 3: Synthesis of ¹¹C-docetaxel

A. Preparation of Docetaxel Primary Amine

The starting material for the preparation of ¹¹C-docetaxel is docetaxel primary amine, which is not available commercially. The free amine is prepared by removal of the tert-butyl carbonyl group from docetaxel using formic acid or other methods known in the art..

10 Docetaxel was purified from its commercial form (TAXOTERE® for Injection RhonePoulencRorer) by silica gel chromatography. Docetaxel (4.5 mg) was dissolved in EtOH (1 ml). After addition of formic acid (1 ml) the mixture was stirred at room temperature for 10 days. Overall conversion to docetaxel primary amine was 48%. Water (2 ml) and methylene chloride (2 ml) were added to the reaction solution and mixed. The
15 aqueous phase containing docetaxel primary amine was separated from the organic phase containing residual docetaxel. The aqueous phase was neutralized with saturated sodium bicarbonate in water. Methylene chloride (12 ml) was added to the aqueous component, mixed and centrifuged. The organic phase, which contained the docetaxel primary amine, was separated and dried with a gentle stream of air at 55°C.

20 B. Preparation of ¹¹C-docetaxel

Docetaxel primary amine (100 µg) was dissolved in ethyl acetate(200 µl) and 0.6 equivalents of di-tert butyl dicarbonate (di-BOC) was added. The solution was heated at about 60°C for 10 minutes. The docetaxel product was produced in 15% yield and its identity verified by HPLC.

25 This rapid, single-step process for preparation of unlabeled docetaxel may be converted to a radioactive method by the use of ¹¹C-labelled di-BOC. The key step is substitution of ¹¹C-CO₂ for unlabeled CO₂. Preparation of di-tert-butyl dicarbonate is described in several publications, including: U.S. Patent No. 5,151,542, entitled, "Process For Preparing Di-Tert-Butyl Dicarbonate" or U.S. Patent No. 5,162,565, entitled, "Process For
30 Preparing Diteriary-Alkyl Dicarbonate".

Example 4: Alternative Synthesis of ^{11}C -docetaxel

In a two-step procedure, 10-acetyl docetaxel is prepared from the paclitaxel primary amine, followed by 10-deacetylation via hydrogen peroxide to yield docetaxel. Paclitaxel primary amine (0.5 mg) was added to ethyl acetate (0.2 ml) containing an equimolar amount
5 of di-tert butyl dicarbonate. The solution was heated at 65°C for 30 minutes to form 10-acetyl docetaxel. Upon heating at 125° for about 6 minutes, 20% conversion of 10-acetyl-docetaxel to docetaxel was obtained in 15% hydrogen peroxide.

As in the preceding example, conversion of this procedure to radio-synthesis requires preparation of ^{11}C -labeled di-BOC.

10 Example 5: Synthesis of ^{11}C -doxorubicin**A. Preparation of Protected Demethylated Doxorubicin**

Protected demethylated doxorubicin, the precursor for the synthesis of radio-labeled doxorubicin was formed by first dissolving doxorubicin (20mg, 36 μmol) in isopropanol (5 ml) and sodium borate (1ml of 0.25M), and reaction with 9-Fluorenylmethyl chloroformate (fmoc) (20 mg, 70 μmol) at room temperature with sonication for 20min. The solution was reduced in volume under a stream of air to ~1 ml, extracted into chloroform, and washed with water(2x). The chloroform was removed under a stream of air, leaving dox-fmoc suitable for the next step.

Dox-fmoc (10 mg) was dissolved in dry pyridine (3 ml) and cooled in an ice bath.
20 Benzoyl chloride (125 μl , 0.9 mmol) was slowly added, and allowed to react for 30 min. The reaction (monitored by HPLC) was quenched by addition of water (0.5ml), and blown down under air (50°C). The reaction mixture was extracted into methylene chloride and washed with water (3x). The methylene chloride was removed under a stream of air, and dried under vacuum, producing dox-fmoc-4bz as identified by HPLC-MS.

25 Dox-fmoc-4bz was dissolved in methylene chloride (about 4ml) and cooled in a dry ice/isopropanol bath. BCl_3 (100-200 μl of 2M in heptane) was added. The reaction was maintained at -78°C for about 2 hours (monitored by LC), and quenched by addition of 2% acetic acid (1 ml) at -78°C, washed with water(2x), and the methylene chloride removed under a stream of air, and dried under vacuum. The 4-OH-dox-fmoc-4bz product was isolated
30 by HPLC, in about 5% yield.

B. Synthesis of ^{11}C -Doxorubicin

Methylation conditions were adapted from Bernardi et al. Bernardi L, Masi P, Spaini O, Suarato A, Arcamone F. 4-Demthoxy-4-alkoxydaunorubicins. *Il Farmaco Ed.Sc.* 1978; 34:884-889. The 4-OH precursor (4-OH-dox-fmoc-4bz) was added to a vial containing AgO (approximately 3 mg) and isopropanol (250 μl). Methyl iodide (10ug, 75nmol) was introduced, the vial was capped and heated at 120°C for about 13 minutes. The reaction mixture was filtered and dried under a stream of air. The protecting groups were removed stepwise, adapting the procedures of Adams. Adams N, Blake C, Broadhurst MJ et al. Synthesis and antitumor activity of novel 4-demethoxyanthracyclines. *J. Med. Chem.* 1990; 33:2375-9. The dry reaction products were dissolved in acetone to which 0.5 volumes of dilute KOH is added, heated to 100°C for about 6 minutes, then neutralized with dilute acetic acid and dried. The reaction products are then redissolved in chloroform and treated with morpholine at 100°C for 5 minutes. Doxorubicin can then be isolated by HPLC.

Radio-labeled doxorubicin is prepared by substituting ^{11}C -methyl iodide for the nonradioactive methyl iodide in the methylation step. One skilled in the art will appreciate that the reaction has been conducted on a scale and with amounts of reagents in the range and within a timeframe suitable for tracer radio labeling with ^{11}C -methyl iodide. Preparation of ^{11}C -methyl iodide is established in the art and a standard reagent at facilities equipped for PET synthesis.

^{11}C -epirubicin may be prepared in an identical manner by substitution of epirubicin for doxorubicin.

Example 6: Synthesis of ^{11}C -topotecan

A. Synthesis of the N-desmethyl topotecan.

The starting material for the preparation of ^{11}C -topotecan, N-desmethyl Topotecan, is not commercially available. The N-desmethyl topotecan is prepared from commercial topotecan through a procedure adapted from the procedure of Rao,et.al. (Rao,P.N., Acosta,C.K., Cessac,J.W., Bahr,M.L., Kim,H.K. Synthesis of N-desmethyl derivatives of 17 a-acetoxy-11b-(4-N,N-dimethylaminophenyl)-19-norpregna-4,9-diene-3,20-dione and mifepristone. *Steroids* 1999; 64:205-212.

To a solution of Topotecan dissolved in 2:1 tetrahydrofuan:methanol (v:v) was added approximately 20 mol eq. CaO, and cooled in an ice bath. 10 mol eq. of I₂ was added. The

reaction was allowed to proceed for 2 hours with periodic vortexing. The reaction was quenched with 10% sodium thiosulfate, acidified with dilute formic acid, the solids removed by centrifugation, and dried. N-Desmethyl topotecan was purified by HPLC. Identity of the N-desmethyl topotecan was confirmed by LC/MS.

5 B. Preparation of ^{11}C -Topotecan

N-desmethyl topotecan was dissolved in dimethylformamide to which methyl iodide was added. The reaction vessel was capped and heated at 100°C, for about 4 minutes. The reaction mixture was concentrated and ^{11}C -Topotecan purified by HPLC.

10 Radio-labeled topotecan is prepared by substituting ^{11}C -methyl iodide for nonradioactive methyl iodide. Preparation of ^{11}C -methyl iodide is established in the art and a standard reagent at facilities equipped for PET syntheses.

Example 7: General Procedures for Clinical Use

The positron-labeled antitumor drug is prepared shortly before use, i.e., within 2 hours of injection, and preferably within less than 1 hour. The positron-labeled antitumor drug with 15 an activity of 1 to 300 mCi, preferably 10-60 mCi, is injected into the patient as an intravenous bolus, i.e., within less than 5 minutes, preferably in 1 minute. The patient is placed in a PET scanner, and images are obtained using standard techniques known to the art at 5- to 10-minute intervals following the injection, up to at least 60 minutes, preferably 90 minutes, when image quality is satisfactory. When ^{18}F or other positron-emitting isotopes 20 with a longer half-life is used, imaging for hours or a day or more may be feasible. Variations and improvements in machine technology may permit even longer imaging periods, which is desirable. It is within the scope of the present invention for an operator skilled in the art of PET scanning to modify the method in such ways as changes and improvements in PET scans require or allow.

25 Example 8: Use of Positron-Labeled Antitumor Drugs for Selection of Chemotherapy

A patient with a tumor is imaged with one or more positron-labeled antitumor drugs according to the procedures in Example 7. One or more of the labeled drugs is injected. If more than one antitumor drug is used, a 90 minute period (or longer) between injections is necessary for ^{11}C labeled drugs. Four or more hours between separate injections may be 30 required if ^{18}F or other isotopes with longer half-lives are used. Based upon the accumulation

of antitumor drug demonstrated in the images, therapy can be guided by whether the patient's tumor is classified as sensitive or resistant to one or more of the drugs.

Example 9: Development of Modulators for Antitumor Drug Delivery to Tumors

The procedures in Example 7 can be used to obtain a baseline evaluation of antitumor drug accumulation within the tumor, in the absence of any modulation attempts. These 5 procedures are then repeated in the presence of a specific modulation strategy, or a series of modulation attempts; and the images are compared to determine success or failure.

Example 10: Combinations of Antitumor Drugs

The procedures used in Example 7 may be used to monitor antitumor drug 10 accumulation in both normal tissue and tumors when combinations of antitumor drugs are used under a variety of dose and temporal adjustments. In practicing the method, one of the antitumor drugs is labeled in order to determine the effect of other antitumor drugs on the delivery of the labeled drug. Alternatively, if the drugs are administered sequentially and non-simultaneously, both of the antitumor drugs of the combination may be labeled in order to 15 determine drug delivery overall.

Example 11: Targeting of Tumor Types for Drug Development

The use of positron-labeled antitumor drugs, following the procedures of Example 7, provide a basis for selecting tumor types for further emphasis in drug development. Indeed, the entire paradigm of drug selection could shift from dominance by histopathologic findings 20 to reliance upon actual accumulation of radio-labeled antitumor drugs to guide initial therapy.

The embodiments illustrated and discussed in this specification are intended only to teach those skilled in the art the best way known to the inventors to make and use the invention. Nothing in this specification should be considered as limiting the scope of the present invention. All examples presented are representative and non-limiting. The above-25 described embodiments of the invention may be modified or varied, and elements added or omitted, without departing from the invention, as appreciated by those skilled in the art in light of the above teachings. It is therefore to be understood that, within the scope of the claims and their equivalents, the invention may be practiced otherwise than as specifically described.

WHAT IS CLAIMED IS:

1. A method of measuring the accumulation of antitumor drugs by solid tumors comprising,

5 administering an antitumor drug labeled with a positron-emitter to a patient having a solid tumor, and

imaging at least part of the patient using PET.

2. The method according to claim 1, wherein the solid tumor is selected from the group consisting of breast, lung, ovarian, gastrointestinal, prostate, sarcoma and head and neck tumors.

10 3. The method of claim 1, wherein the labeled drug is at least one drug selected from the group consisting of ^{11}C -paclitaxel, ^{11}C -docetaxel, ^{11}C -doxorubicin, ^{11}C -epirubicin, ^{11}C -mitoxantrone, ^{11}C -topotecan, and a drug for the treatment of solid tumors that has been 15 radiolabeled.

4. A method of determining the efficacy of an antitumor drug for treating solid tumors comprising:

20 administering an antitumor drug labeled with a positron-emitter to a patient having a solid tumor; and

imaging at least part of the patient by PET to measure accumulation of the labeled antitumor drug.

25 5. The method according to claim 4, wherein the labeled antitumor drug is administered prior to a course of treatment of the patient.

6. The method of claim 4, wherein the labeled antitumor drug is administered during the course of treatment of the patient.

30 7. The method of claim 4, wherein the labeled drug is at least one drug selected from the group consisting of ^{11}C -paclitaxel, ^{11}C -docetaxel, ^{11}C -doxorubicin, ^{11}C -epirubicin,

¹¹C-mitoxantrone, ¹¹C-topotecan, and a drug for the treatment of solid tumors that has been radiolabeled.

8. A method of measuring the effectiveness of modulators of cellular
5 accumulation mechanisms in tumors comprising:
administering an antitumor drug labeled with a positron-emitter to a patient;
administering a modulator to the patient, and
imaging at least part of the patient by PET to measure accumulation of the labeled
antitumor drug.

10 9. The method of claim 8, wherein the accumulation of labeled antitumor drug
is measured before and after administering the modulator to the patient and the levels of
antitumor drug accumulation before and after administering the modulator are compared.

15 10. The method of claim 8, wherein modulator affects an efflux mechanism.

11. The method of claim 8, wherein modulator affects an influx mechanism.

12. The method of claim 8, wherein the labeled drug is at least one drug selected
20 from the group consisting of ¹¹C-paclitaxel, ¹¹C-docetaxel, ¹¹C-doxorubicin, ¹¹C-epirubicin,
¹¹C-mitoxantrone, ¹¹C-topotecan, and a drug for the treatment of solid tumors that has been
radiolabeled.

13. A method for measuring the effectiveness of a combination of antitumor
25 drugs comprising:

administering more than one antitumor drug to a patient having a solid tumor,
wherein at least one of said antitumor drugs is labeled with a positron-emitter, and
imaging at least part of the patient by PET to measure accumulation of the labeled
antitumor drug.

30 14. The method of claim 13, wherein two antitumor drugs are administered to
the patient.

15. The method of claim 13, wherein one of said antitumor drugs is labeled with a positron-emitter.

5 16. The method of claim 13, wherein two of said antitumor drugs are each labeled with a positron-emitter.

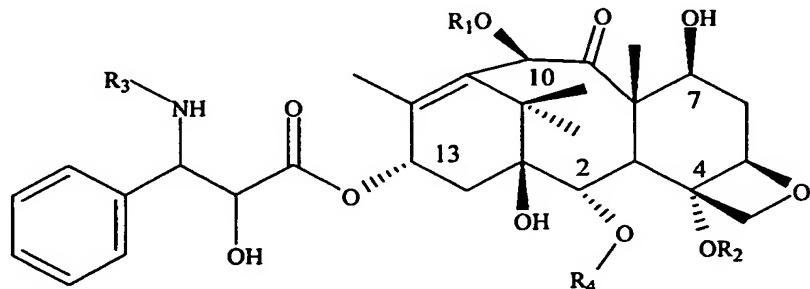
17. The method claim 13, wherein a first antitumor drug and a second antitumor drug are administered simultaneously.

10

18. The method claim 13, wherein a first antitumor drug and a second antitumor drug are administered sequentially.

19. The method of claim 13, wherein the labeled drug is at least one drug selected from the group consisting of ^{11}C -paclitaxel, ^{11}C -docetaxel, ^{11}C -doxorubicin, ^{11}C -epirubicin, ^{11}C -mitoxantrone, ^{11}C -topotecan, and a drug for the treatment of solid tumors that has been radiolabeled.

20. A compound having the formula:



20 wherein:

R₁ is selected from the group consisting of H, acetate and ^{11}C -acetate;

R₂ is selected from the group of acetate and ^{11}C -acetate;

R₃ is selected from the group consisting of benzoyl, ^{11}C -benzoyl, $-\text{CO}_2\text{C}(\text{CH}_3)_3$ and $-^{11}\text{CO}_2\text{C}(\text{CH}_3)_3$; and

25 R₄ selected from the group consisting of benzoyl ^{11}C -benzoyl; and

wherein the compound contains at least one atom of ^{11}C .

21. A compound according to claim 20, wherein R_1 is ^{11}C -acetate, R_2 is acetate, R_3 is benzoyl and R_4 is benzoyl.

5

22. A compound according to claim 20, wherein R_1 is acetate, R_2 is ^{11}C -acetate and R_3 is benzoyl and R_4 is benzoyl.

23. A compound according to claim 20, wherein R_1 and R_2 are acetate and R_3 is
10 ^{11}C -benzoyl and R_4 is benzoyl.

24. A compound according to claim 20, wherein R_1 and R_2 are acetate, R_3 is
benzoyl and R_4 is ^{11}C -benzoyl

15 25. A compound according to claim 20, wherein R_1 is H, R_2 is acetate, R_3 is
 $^{11}\text{CO}_2\text{C}(\text{CH}_3)_3$ and R_4 is benzoyl.

26. A compound according to claim 20, wherein R_1 is H, R_2 is ^{11}C -acetate, R_3 is
 $\text{CO}_2\text{C}(\text{CH}_3)_3$ and R_4 is benzoyl

20

27. A compound according to claim 20, wherein R_1 is H, R_2 is acetate, R_3 is
 $-\text{CO}_2\text{C}(\text{CH}_3)_3$ and R_4 is ^{11}C -benzoyl.

28. A method of synthesizing the compound according to claim 20, comprising
25 the steps of:

reacting 10-deacetylpaclitaxel with a chlorotrialkylsilane to yield a protected
deacetylpaclitaxel;

reacting the protected deacetylpaclitaxel with ^{11}C -acetyl chloride to yield a radio-
labeled silyl protected deacetylpaclitaxel; and

30 removing the protecting groups to produce ^{11}C -paclitaxel.

29. A method of synthesizing the compound according to claim 20, comprising the step of:

reacting paclitaxel primary amine with ^{11}C -benzoyl chloride to produce ^{11}C -paclitaxel.

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30. A method of synthesizing the compound according to claim 20, comprising the step of:

reacting docetaxel primary amine with ^{11}C -di-tert-butyl dicarbonate to produce ^{11}C -docetaxel.

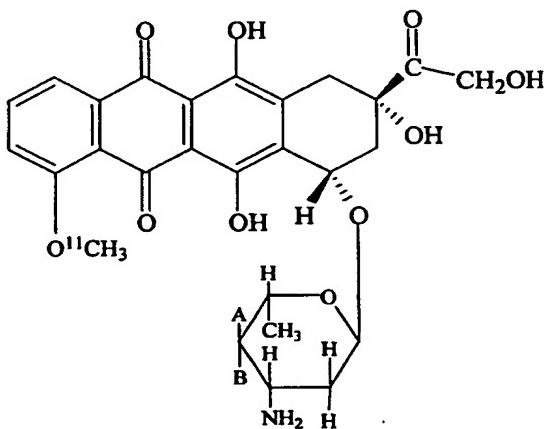
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31. A method of synthesizing the compound according to claim 20, comprising the steps of:

reacting paclitaxel primary amine with ^{11}C -di-tert-butyl dicarbonate to give ^{11}C -10-acetyldocetaxel; and

15 reacting the ^{11}C -10-acetyldocetaxel with hydrogen peroxide to produce ^{11}C -docetaxel.

32. A compound having the formula:



20 wherein one of A or B is H and the other of A or B is OH.

33. The compound of claim 32, wherein A is H and B is OH.

34. The compound of claim 32, wherein A is OH and B is H.

35. A method of synthesizing the compound according to claim 32, comprising the steps of:

5 protecting the amino group of doxorubicin with an amino protecting group to yield an amino-protected doxorubicin;

protecting the hydroxy groups of the amino-protected doxorubicin with one or more hydroxy protecting groups to yield a fully protected doxorubicin;

demethylating the aromatic methoxy group to yield a 4-OH protected doxorubicin;

10 reacting the 4-OH protected doxorubicin with ^{11}C -methyl iodide; and

removing the amino and hydroxy protecting groups to produce ^{11}C -doxorubicin.

36. The method according to claim 35, wherein the amino protecting group is 9-fluorenylmethyl, the hydroxy protecting group is benzoyl, and the fully protected

15 doxorubicin is demethylated using boron trichloride.

37. A method of synthesizing the compound according to claim 32, comprising the steps of:

20 protecting the amino group of epirubicin with an amino protecting group to yield an amino-protected epirubicin;

protecting the hydroxy groups of the amino-protected epirubicin with one or more hydroxy protecting groups to yield a fully protected epirubicin;

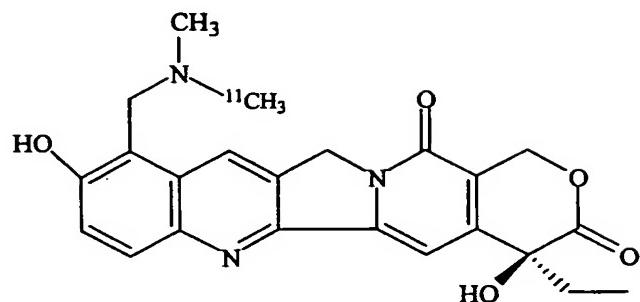
demethylating the aromatic methoxy group to yield a 4-OH protected epirubicin;

reacting the 4-OH protected epirubicin with ^{11}C -methyl iodide; and

25 removing the amino and hydroxy protecting groups to produce ^{11}C -epirubicin.

38. The method according to claim 35, wherein the amino protecting group is 9-fluorenylmethyl, the hydroxy protecting group is benzoyl, and the fully protected epirubicin is demethylated using boron trichloride.

39. A compound having the formula:



40. A method of synthesizing the compound according to claim 39, comprising
the step of:

5 reacting N-desmethyl topotecan with ¹¹C-methyl iodide to produce ¹¹C-topotecan.